TOXICITY OF A *MICROCYSTIS* WATERBLOOM FROM AN OHIO POND[1]

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ABSTRACT

A toxin extracted from a waterbloom of the blue-green alga *Microcystis aeruginosa* was found to have an LD_{100} to mice of 3 mg/kg. Death occurred in less than 4 hours and was accompanied by symptoms corresponding to those of the fast death factor found by others in blue-green algae. The toxin was found to have no effects on three species of fish and a microcrustacean in concentrations up to 10 mg/l, and showed no growth-inhibitory effects on a diatom.

INTRODUCTION

In August, 1964, a heavy waterbloom of algae was observed in a small pond in the Mt. Washington section of Cincinnati, Ohio. The waterbloom was found to be almost entirely composed of the blue-green alga *Microcystis aeruginosa* Kutz. emend. Elenkin (*Anacystis cyanea* Drouet and Daily). Concurrent with the waterbloom, large numbers of dead fish were seen in the pond. It could not be determined, however, how long the fish had been dead and whether their death was due to anoxia caused by bacterial decomposition of the algae or to a toxin liberated by the algae.

The toxic action of blue-green algae, including this species, has been reported to cause illness and/or death in mammals, birds, and fish (Prescott, 1948; Ingram and Prescott, 1954; Schwimmer and Schwimmer, 1964). *Microcystis aeruginosa* has, in fact, been one of the species of blue-green algae that has received considerable attention in laboratory studies of algal toxins (Wheeler et al., 1942; Gorham, 1962; Grant and Hughes, 1953; Bishop et al., 1959; Hughes et al., 1958; Gorham, 1964). Toxic and non-toxic strains have been discovered. With its associated bacteria, a toxic strain of this alga was found to produce two distinguishable toxic factors to white mice (Hughes et al., 1958). One factor, the fast death factor (FDF), resulted in the death of mice about one hour after oral or intraperitoneal injection. The other factor, the slow death factor (SDF), caused death within 4 to 48 hours after administration. Although the FDF tended to obscure the presence of the SDF, the two factors could be separated because FDF was found to be an endotoxin. The conclusion was made that the alga was the source of FDF and the bacteria were the source of SDF. The isolation and identification of the FDF in *Microcystis aeruginosa* (Bishop et al., 1959) showed that the toxin was a peptide, probably cyclic in nature, containing seven amino acids.

The toxicity of blue-green algae to aquatic animals was discussed in a review (Gorham, 1962) in which it was pointed out that only a few of the toxic effects on these animals have been adequately corroborated or systematically investigated. Evidence was presented that suggested the possibility of fish deaths being caused by decomposing *Aphanizomenon flos-aquae* in tanks, aquaria, and ponds containing a variety of species of fish (Prescott, 1948). After chemical analysis of the alga, however, hydroxylamine was found to be present in sufficient quantity to kill fish. Also, quantitative tests showed 8.5 mg/l of hydrogen sulfide to be given off by decomposing algae. In this case, death of the fish was apparently not due to an algal toxin, but to a poisonous product resulting from bacterial decomposition

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of the algae. Other workers conducted experiments concerning the effects of decomposing *Aphanizomenon flos-aquae* on a variety of fish (Mackenthun et al., 1948) and concluded that, although the fish died, the primary cause of fish mortality was the depletion of oxygen supply brought about by decaying algae, and the secondary cause of death was the presence of toxic elements released by the decomposing algae. Suspensions of living cells of *Microcystis aeruginosa* were injected intraperitoneally into carp ranging in weight from 8 to 500 g (Shelbusky, 1950); the minimal lethal doses ranged from 1 mg for the smaller fish to 50 mg for the larger fish. Death occurred 36 to 96 hours after injection. A scum of *Anabaena* and *Aphanizomenon*, collected at a dam which supplies water to a small city in Saskatchewan, Canada, was reported to have immobilized *Daphnia* within 30 minutes of administration (Dillenberg and Dehnel, 1960). After passage through 5 miles of pipeline to the water plant, aliquots of the scum did not immobilize *Daphnia* within 52 hours.

The study described herein is concerned with determining whether the algal waterbloom contained toxic strains of *Microcystis* and, if so, whether the toxin produced had deleterious effects on fish and other aquatic organisms as well as on mice.

MATERIALS AND METHODS

Preliminary Tests

Approximately 1 gallon of the waterbloom was skimmed from the surface of the pond, placed in a polyethylene container, and almost immediately placed in a deep freeze. The toxicity of the waterbloom was tested by intraperitoneal injection of 1 ml of the thawed material into each of five 19- to 21-g female white mice. Control mice were injected with the same amount of physiological saline solution. All mice injected with the waterbloom died within 1.2 to 2.3 hours. The syndrome of symptoms was similar to that reported for the *Microcystis* FDF (Wheeler et al., 1942; Gorham, 1962), including convulsive jumping, dragging of hind legs, loss of equilibrium, and increasing pallor of ears, eyes, and tail. A brief period of heavy spasmodic breathing immediately preceded death. Mice injected with the same amount of waterbloom, but protected with *Botulinum C.* antisera, died in approximately the same length of time with the same symptoms.

Extraction of Toxin

The freezing and thawing of the waterbloom caused rupture of most of the algal cells. Since FDF is an endotoxin, the toxin could therefore not be concentrated by the recommended procedure (Bishop et al., 1959) of harvesting the cells and freeze-drying them. The toxin is soluble in methanol, *n*-butanol, ethanol, and, as a sodium salt, in water. Because of its immiscibility with water, *n*-butanol was added to 1 liter of the waterbloom and shaken vigorously with a mechanical shaker; the butanol layer was then separated from the aqueous portion. The extraction was repeated five times to insure more extensive extraction. The combined butanol extracts were evaporated to dryness under reduced pressure at room temperature, redissolved in distilled water, and subsequently extracted with ethyl ether and ethyl acetate to reduce colored material. The aqueous phase was then taken to dryness under reduced pressure.

Bioassay of Toxin

The approximate toxicity of the extract was established by intraperitoneal injection of 0.5 ml of each of a series of aqueous dilutions into 19- to 21-g female white mice. For establishing the LD_{100}, 10 mice were injected with each dilution. Sterile syringes and needles were used throughout, but dilutions were prepared with unsterilized water. Control mice received a 0.5-ml intraperitoneal injection of the dilution water.

Testing Toxicity to Aquatic Organisms

The extract was tested for its toxicity to three species of fish, fathead minnows (*Pimephales promelas*), carp (*Cyprinus carpio*), and guppies (*Lebistes reticulatus*), and to the microcrustacean *Daphnia magna*. The procedure used was similar to that recommended for evaluating the toxicity of industrial wastes to fish (Standard Methods for the Examination of Water and Wastewater, 1960). The guppies tested were all adult males; the carp were from 90 to 100 mm long; and the *Daphnia magna* were from 1 to 6 hours old. The guppies and *Daphnia* were tested in 250 ml beakers containing 200 ml of test solution, the fathead minnows in 600 ml beakers containing 400 ml of test solution, and the carp in wide-mouth jars containing 1000 ml of test solution. Aqueous solutions of the extract were added to dilution water in the respective test containers to give a series of concentrations ranging up to 10 mg/l. The test-dilution water was a mixture of spring and distilled water having a versenate hardness of 150 mg/l and a pH of 8.2. The guppies, fathead minnows, and carp were acclimatized to the dilution water for one week prior to testing and the *Daphnia magna* were hatched from adults acclimatized in the same manner. Four guppies, four fathead minnows, two carp, and 20 *Daphnia* were placed in each separate test solution and observed periodically for at least 96 hours. Control organisms were held in dilution water without toxin. The dissolved oxygen contents of the test solutions were maintained above 8.0 mg/l by aeration, and the tests were carried out at 22°C.

The toxin was also tested for its effect upon the growth of the diatom, *Navicula minima*, by the agar-filter paper-disc technique, which has been employed to test the effect of antibiotics upon algal growth (Foter et al., 1953). This was accomplished by placing various concentrations of the toxin, ranging up to 0.4 mg, on 12.7-mm-diameter filter-paper discs, which had been placed on agar plates containing a growth medium and newly seeded with an actively growing culture. The plates were incubated under fluorescent illumination of 350 ft-c and observed for inhibition of diatom growth around the impregnated filter-paper discs for 10 days.

RESULTS AND DISCUSSION

The butanol extract from 1 liter of waterbloom yielded 0.5 g of dried material having an LD_{100} to mice of 3 mg/kg. Time of death ranged from 0.9 to 3.5 hours, with the average being 1.6 hours. The symptoms were identical to those observed in the preliminary tests. If death did not occur in 3.5 hours, the mice survived for at least 36 hours. This suggests that the extract contained the FDF ordinarily attributed to blue-green algae and that the SDF associated with bacterial contaminants was either initially absent or eliminated by the extraction method.

The extract, in concentrations up to 10 mg/l, was not toxic to the three species of fish nor to *Daphnia magna*. Although the limited amount of available extract precluded testing these organisms with higher concentrations, the results suggest that these aquatic forms are relatively unaffected when exposed to *Microcystis* FDF in their environment and that death of the fish in the pond was due to other causes such as anoxia. Intraperitoneal injection of live *Microcystis* cells into carp was reported to cause death in 36 to 96 hours, with lethal doses ranging from 1 to 50 mg, depending upon the size of the fish (Shelubsky, 1950). In view of the difference in the testing methods employed in that study and in this, a comparison of results can not adequately be made. Because intraperitoneal injection of both live cells of a toxic strain of *Microcystis* and *Microcystis* FDF into mice caused rapid death (Hughes et al., 1958; Gorham, 1962), it appears that, when tested in the same manner, *Microcystis* is much less toxic to carp then to mice.

Others (Dillenberg and Dehnel, 1960) reported that a scum containing *Anabaena* and *Aphanizomenon* immobilized *Daphnia* within 30 minutes. No mention, how-

ever, was made of the dissolved oxygen content of the water. The fact that the scum was not capable of immobilizing *Daphnia* within 52 hours after passage through a 5-mile pipeline suggests that perhaps the oxygen content of the original test water was depleted and pumping and passage through the pipeline raised the oxygen content to a level adequate for the survival of *Daphnia*.

The extract did not display any growth-inhibitory effects on the diatom, *Navicula minima*. Others (Bishop et al., 1959) reported that *Microcystis* FDF had no antibiotic effects against four strains of bacteria.

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